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研究課題名（和文）Development of hybrid multi-stage constructed wetlands based on indigenous adsorptive materials for sustainable heavy metal treatment from mine drainages in Japan

研究課題名（英文）Development of hybrid multi-stage constructed wetlands based on indigenous adsorptive materials for sustainable heavy metal treatment from mine drainages in Japan

研究代表者

NGUYEN THI・THUONG (Nguyen Thi, Thuong)

立命館大学・立命館アジア・日本研究機構・研究員

研究者番号：00979627

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研究成果の概要（和文）：本研究では、鉱山排水中の重金属除去を目的に、カルシウムを多く含む養殖副産物・ハマグリ殻を用いた人工湿地を開発しました。2023年4月～2025年3月の実験により、マンガン（83.6～92.3%）、亜鉛（99.4～100%）などの高い除去効果を確認しました。本技術は持続可能で低コストな処理法として有望であり、成果は国内外の学会で発表され、「Excellent Oral Presentation AwardとYoung Water Professional Award」の賞を受賞し、滋賀県の地域住民とも共有されました。

研究成果の学術的意義や社会的意義

This study fills key gaps in heavy metal removal in CWs by revealing removal mechanisms, microbial roles, and metal accumulation. It also supports effluent compliance, reduces costs, and promotes CW adoption. Results were shared with Kinrin residents to raise water conservation awareness.

研究成果の概要（英文）：This study aims to develop a constructed wetland (CW) using indigenous adsorptive materials to enhance heavy metal removal from acid mine drainage (AMD) in Japan. Conducted over two years (April 2023-March 2025), this study selected clamshells, a calcium-rich aquaculture by-product as the main substrate. Planted with common reed, two hybrid CWs filled with crushed clamshells effectively neutralized AMD and achieved high removal efficiencies for Mn (83.6-92.3%), Zn (99.4-100%), and other metals (97.8-100%). The results demonstrate that clamshell-based CWs offer a sustainable, low-cost solution for heavy metal removal while promoting beneficial reuse of aquaculture waste in AMD treatment. The research results were presented at 04 international conferences and 01 domestic conference, earning an Excellent Oral Presentation Award and a Young Water Professional Award. The findings were also shared with residents of Shiga Prefecture at the Water Conservation Symposium.

研究分野：Wastewater treatment

キーワード：Constructed wetlands Heavy metals Acid mine drainages Clamshells Adsorptive materials

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1. 研究開始当初の背景

In Japan, there are approximately 100 abandoned mine drainage sites with no responsible owners. These sites discharge wastewater with high concentrations of heavy metals and low pH levels, requiring continuous treatment despite the cessation of mining activities. This situation imposes a significant economic burden on both national and local governments. Constructed wetlands (CWs) have emerged as a promising passive treatment technology for mine drainage due to their simple operation, low cost, and ecological benefits, including high biodiversity value.

It is well recognized that heavy metals in CWs are primarily removed through interactions with substrates and plant uptake. Therefore, selecting substrates and plant species with high adsorption and uptake capacities is essential. To further minimize the cost of CW systems, substrates should ideally be inexpensive and locally available materials.

In recent years, the expansion of thermal power plants, agriculture, and the marine shellfish industry has led to the generation of coal slag, rice husks, and seashells as by-products, posing challenges in solid waste management. Recycling these materials into useful resources could reduce their environmental impact. Notably, coal slag and seashells are rich in metal oxides and calcium carbonate (CaCO₃), respectively, while rice husks contain significant amounts of fiber and functional groups such as carboxyl and hydroxyl. Several previous studies have used these materials as adsorbents for removing phosphorus, metals, and toxic gases. However, limited research has explored their use as substrates in CWs. Therefore, developing CWs using such materials to enhance metal removal is of great significance.

This study aimed to screen potential agro-industrial by-products as substrates and to identify suitable plant species for use in CWs to enhance heavy metal removal from mine wastewater. The goal is to develop a hybrid multi-stage CW system incorporating indigenous adsorptive materials to improve the treatment efficiency of mine drainage.

The research was conducted over a two-year period (April 2023 – March 2025). In the first year (FY 2023), five materials—corn cob biochar, rice husk, coal slag, clamshells, and limestone—were tested as substrates for treating acid mine drainage (AMD) containing high levels of manganese (Mn) and zinc (Zn). Among them, clamshells, a by-product of the seafood industry, demonstrated high adsorption capacity, excellent hydraulic conductivity, and effective AMD neutralization. They were therefore selected for the development of hybrid CWs. In addition, common reed (*Phragmites australis*), which showed the highest Mn removal efficiency, was chosen as the primary plant species for these systems. In the second year (FY 2024), two-stage CWs were designed and operated to evaluate their treatment performance for Mn- and Zn-rich acid mine drainage.

2. 研究の目的

This study aims to evaluate the performance of a two-stage horizontal subsurface flow constructed wetland (HSSF-CW) filled with clamshells for treating Mn- and Zn-rich mine drainage in Japan. Specifically, it investigates the effects of hydraulic retention time (HRT) and plant presence on the removal efficiency of Mn and Zn. The findings from this study will contribute to the optimization of CW design and operation for mine drainage treatment, offering practical insights for developing nature-based solutions to mitigate heavy metal.

3. 研究の方法

Simulated mine wastewater was prepared based on the chemical composition of effluent from an actual mine in the Tohoku region, Japan. This effluent is characterized by elevated concentrations of Mn and Zn, which significantly exceed the Japanese regulatory discharge limits of 10 mg/L and 2 mg/L, respectively. Additionally, the pH of the wastewater was slightly below the acceptable effluent standard range of 5.8–8.6, as presented in Table 1.

Table 1: Chemical composition of AMD and effluent standards in Japan

Mental	Chemicals	Concentrations (mg/L)	Japan standard (mg/L)	Effluent
Mn	MnSO ₄ •5H ₂ O	66.25	10	
Fe	FeSO ₄ •7H ₂ O	9	10	

Zn	ZnCl ₂	11.73	2
Cu	CuSO ₄ •5H ₂ O	0.02	3
Pb	PbCl ₂	0.11	0.1
Cd	CdCl ₂	0.02	0.03
pH		5	5.8-8.5

Two parallel two-stage CWs filled with clamshells (3-7 mm, 45% porosity, 90% CaCO₃) were prepared (as shown in Fig. 1), one planted with common reeds (P1, P2) and the other unplanted (UP1, UP2).

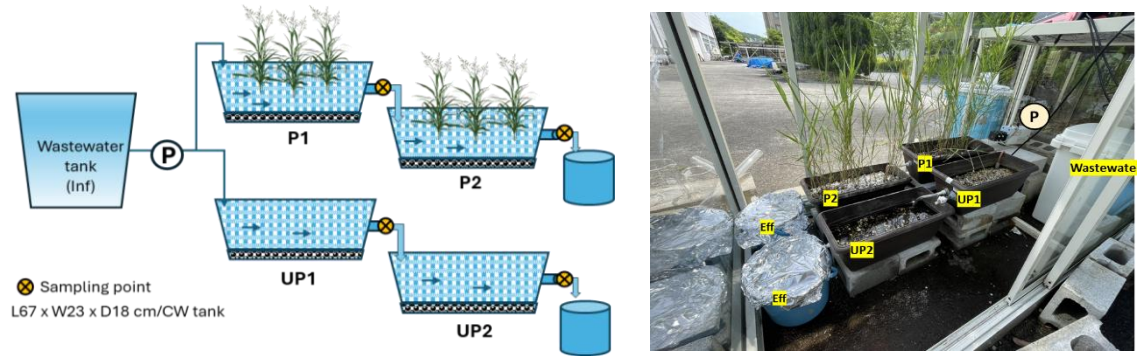


Fig1: Diagram of the treatment system.

Synthetic AMD, containing 67.3 mg/L of Mn, 10.6 mg/L of Zn, and other minerals, with a pH of 5.3, was continuously fed to the CWs in four phases (I-IV) with HRTs of 48 h, 24 h, 12 h, and 48 h, respectively. Water samples were collected to analyze heavy metals and other parameters. Mn-oxidizing (MnOB) and sulfate-reducing bacteria (SRB) were detected using modified AY medium [1] and Postgate's medium F [2], respectively. At the end of the experiment, microorganisms in sediment samples were detected using DNA extraction and Illumina MiSeq 16S rRNA gene sequencing.

4. 研究成果

During the experiment, clamshells with high CaCO₃ content effectively neutralized AMD, raising the effluent pH to 7.4–8.4. All CWs efficiently removed Mn (94.0–95.0%), Zn (99.4–100%), and other metals such as Cd, Cu, Fe, and Pb (97.8–100%), maintaining stable removal across all HRTs, although Mn slightly re-eluted in Phase IV. Effluent concentrations of Mn (0–9.4 mg/L), Zn (0–0.7 mg/L), and other metals met Japan's discharge standards (Fig. 2).

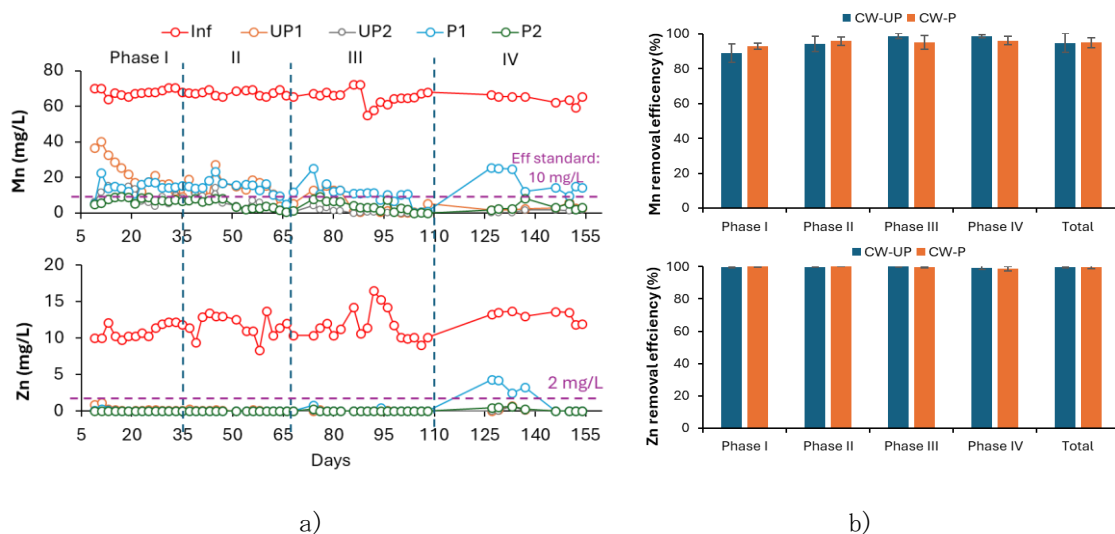


Fig.2: (a) Mn and Zn concentrations during the experiment, (b) Mn and Zn removal efficiency

Vegetation contributed significantly to Mn removal in Phase I, but its effectiveness decreased in Phases II-IV. Notably, MnOB (Fig. 3b) and SRB were detected in both systems, potentially promoting

the precipitation of Mn oxides and metal sulfides. At the genus level (Fig. 3a), the proportion of *Bacillus* was high in P1 and P2. Various genera such as *Nitrospira* and *Hydrogenophaga* were identified in UP1 and UP2, which are microorganisms involved in nitrogen metabolism and hydrogen metabolism. UP1 and UP2 tend to have higher microbial community diversity than P1 and P2.

The Shannon index diversity of microorganisms in P1, P2, UP1, and UP2 was 4.54, 4.92, 4.07, and 4.21, respectively, falling under the high diversity category. This indicates that the two-stage clamshell-based CWs created diverse environmental conditions that enhanced microbial diversity, leading to more efficient treatment of Mn- and Zn-rich AMD. Among the dominant bacteria in CWs, *Bacillus* (0.3–18.1%), *Sphingopyxis* (0–1.1%), *Nocardioides* (0.1–1.1%), and *Rhodobacter* (0.5–1.4%) are known as Mn-oxidizing bacteria genera. These bacteria use oxygen to oxidize Mn(II) to Mn(IV) under aerobic conditions. The Mn(IV) formed through oxidation precipitates as Mn oxides (MnO₂), which are insoluble in water and can be easily removed through filtration, sedimentation, or adsorption to surfaces within CWs. Additionally, these bacteria promote Zn removal by adsorbing or co-precipitating it with Mn oxides.

Furthermore, genera such as *Chroococcus* (0–15.0%), *Leptolyngbya* (1.8–25.1%), *Rhodoplanes* (0.4–1.3%), *Chloronema* (0.6–2.7%), *Acutodesmus* (0–5.2%), and *Calothrix* (0–2.4%) possess photosynthetic capabilities, producing oxygen during photosynthesis. This oxygenation helps maintain an aerobic environment, which supports Mn oxidation, leading to the formation of MnO₂ that precipitate and can be filtered out, thereby facilitating Mn removal. While Zn is primarily removed through adsorption onto Mn oxides, increased oxygen levels also support aerobic metal-oxidizing bacteria, which contribute to Zn removal by promoting its precipitation as Zn hydroxides (Zn(OH)₂) or through complexation with other precipitates. Additionally, during photosynthesis, these bacteria utilize CO₂ from the environment, reducing CO₂ concentration and increasing pH.

Other aerobic bacteria, such as *Bosea* (0–1.1%) and *Pseudonocardia* (0–2.2%), contribute to Mn oxidation, while *Gemmata* (0.7–3.0%) and *Hyphomicrobium* (0–1.4%) enhance biofilm formation, allowing efficient biosorption of metals such as Mn, Zn, Fe, and Cu. *Hyphomicrobium* also aids in organic matter degradation, improving overall treatment efficiency.

In contrast, *Clostridium* (0.2–5.5%) and *Pseudoxanthomonas* (0.2–1.2%) are known Mn-reducing bacteria. These bacteria also facilitate metal sulfide formation through interactions with sulfate-reducing bacteria, which aids in the removal of metals like Zn and Cd. Additionally, they promote the creation of anaerobic zones, supporting the activity of other metal-reducing bacteria.

Sulfate-reducing bacteria, including members of the *Desulfovibrionaceae*, *Desulfomicrobiaceae*, *Desulfobulbaceae*, and *Thermodesulfovibrionaceae* families, accounted for 0.1–1.0% of the bacterial community in all CWs, with the highest population (1.0%) observed in UP1. SRBs reduce sulfate, leading to the precipitation of metals such as Zn, Cd, Cu, Fe, and Pb as metal sulfides. By neutralizing acidity and forming biofilms, SRBs significantly improve water quality while contributing to a sustainable and passive remediation system for acid AMD-contaminated sites.

In addition, the Mn and Zn concentrations correlated negatively with pH values, implying that Zn was removed as the hydroxide and adsorbed onto the Mn oxide.

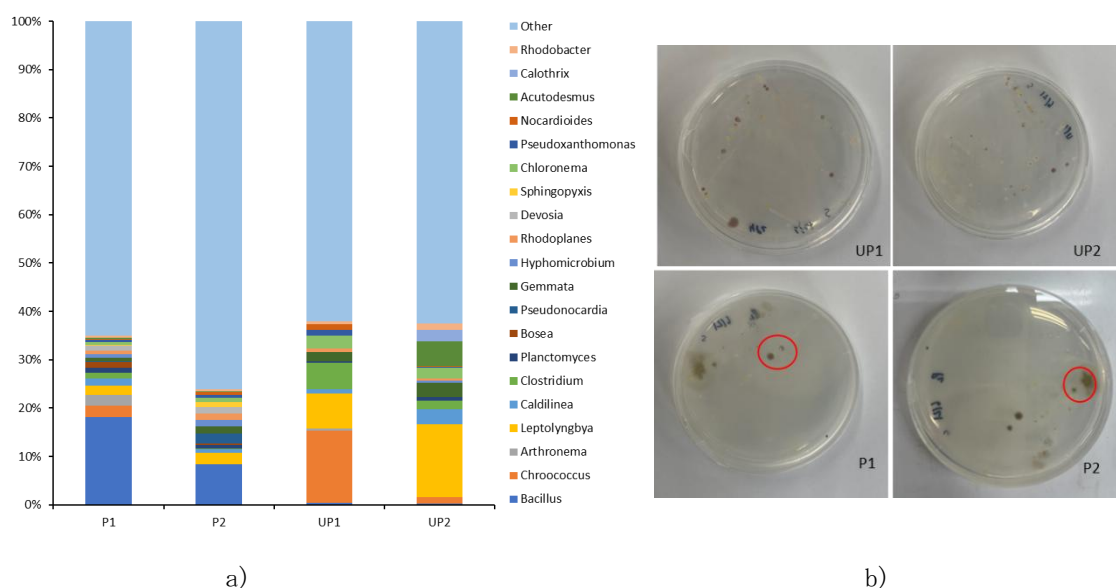


Fig.3: (a) Relative abundance of bacteria in each CW at genus level, and (b) MnOB detected in CWs.

Generally, the two-stage CWs filled with clamshells demonstrated high treatment performance for

Mn- and Zn-rich AMD, even at a short HRT of 12 hours. The sequential treatment in two stages enhances metal removal efficiency by creating different environmental conditions that facilitate oxidation, adsorption, and precipitation processes. In the first stage, the dissolution of CaCO_3 from the clamshells increases pH and alkalinity, promoting the precipitation of metal hydroxides under elevated pH conditions. This stage also effectively removes other metals such as Fe and Cu, reducing their interference in the subsequent treatment stage. By eliminating these metals early, the second stage can focus on Mn and Zn removal through microbial oxidation and further hydroxide precipitation. The two-stage design also helps minimize clogging by distributing metal deposits across both stages, thereby extending the operational lifespan of the CWs. Zinc is primarily removed through sorption and co-precipitation, while manganese oxidation is enhanced under controlled redox conditions, improving overall treatment efficiency and stability.

Common reed (*Phragmites australis*) played a significant role in heavy metal removal during the initial phase but was less effective in the later stages. This study presents an ecological approach that not only reduces the environmental burden of seashell waste but also lowers the cost of wastewater treatment.

References

- [1] N. Miyata, Y. Tani, K. Iwahori, M. Soma: FEMS Microbiol. Ecol., 47(1), pp. 101-109 (2004).
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5. 主な発表論文等

〔雑誌論文〕 計0件

〔学会発表〕 計5件（うち招待講演 0件 / うち国際学会 4件）

1. 発表者名 Nguyen Thi Thuong
2. 発表標題 Heavy Metal Removal from Simulated Acid Mine Drainage Using Clamshell- and Gravel-Filled Lab-scale Constructed Wetlands
3. 学会等名 IWA conference (国際学会)
4. 発表年 2023年

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2. 発表標題 Seashell-Enhanced Constructed Wetlands: A Sustainable Solution for Mine Drainage Treatment
3. 学会等名 Asia Pacific Conference 2024 (国際学会)
4. 発表年 2024年

1. 発表者名 Nguyen Thi Thuong
2. 発表標題 Development of two-stage constructed wetlands filled with clamshells for mine drainage treatment
3. 学会等名 VANJ conference 2024 (国際学会)
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2. 発表標題 The use of constructed wetlands for wastewater treatment
3. 学会等名 Japan-Philippines YWP Symposium 2024 (国際学会)
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1. 発表者名 Nguyen Thi Thuong
2. 発表標題 Treatment of Mn- and Zn-Rich Mine Drainage Using Two-Stage Clamshell-Based Constructed Wetlands
3. 学会等名 Japan Society on Water Environment 2025
4. 発表年 2025年

〔図書〕 計0件

〔産業財産権〕

〔その他〕

<p>Riceball seminar https://www.ritsumeai.ac.jp/research/ro_oic/event/?event_id=62。 WFPW国際協力シンポジウム「世界水の日」によせて」、滋賀県、2025年2月24日。</p>

6. 研究組織		
氏名 (ローマ字氏名) (研究者番号)	所属研究機関・部局・職 (機関番号)	備考

7. 科研費を使用して開催した国際研究集会

〔国際研究集会〕 計0件

8. 本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関
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